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New Mechanism of Regulation of K+-Cl- Cotransport

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NEW MECHANISM OF REGULATION OF K^+-Cl^- COTRANSPORT. ((**N.C. Adragna**, H. Ahmed, C.M. Ferrell, J. Filosa and P.K. Lauf)) Wright State University, Dayton, OH 45435.

Nitrovasodilators, through a cGMP-dependent pathway involving the inositol phosphate (IP) cycle decrease intracellular Ca^{++} and induce vasodilation by activation of ion channels. K^+-Cl^- cotransport (COT) promotes the electroneutral-coupled movement of K^+ and Cl^- ions and plays an important role in regulatory volume decrease. K^+-Cl^- COT is activated by cell swelling, thiol (SH) reagents, and by lowering cellular Mg^{++} and H^+ concentrations. Our recent findings indicate that Li regulates K^+-Cl^- COT with involvement of the IP cycle and that in low K sheep red blood cells (LK SRBCs), nitrite (a nitric oxide derivative) and other vasodilators stimulate the transporter from two- to 10-fold. To test our hypothesis whereby a new mechanism of regulation of K^+-Cl^- COT involves the cGMP-dependent pathway, we further characterized the effect of the vasodilators studied both in LK SRBCs and in cultured vascular smooth muscle cells (CVSMCs). K^+-Cl^- COT was measured at different osmolalities, drug concentrations and in the presence of inhibitors of other pathways for K(Rb) transport. Hydralazine stimulated K-Cl COT by 5-fold in LK SRBCs and by 20-fold in CVSMCs, and was the most potent activator in LK SRBCs where it decreased glutathione by 100%. Protein/threonine phosphatase 1 and tyrosine kinase regulate K^+-Cl^- COT via a metabolic cascade. Inhibition of the phosphatase and kinase by calyculin and genistein, respectively, suggest an upstream site for the action of hydralazine and isosorbide mononitrate. Further testing of our hypothesis include the study of specific activators and inhibitors of the cGMP pathway on K^+-Cl^- COT activity. Our findings suggest a role for K^+-Cl^- COT in vasodilation. (Supported by NIH, NAHA, AHAOH and the WSU BMS Program)

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NEW METABOLIC PATHWAYS IN THE REGULATION OF THE SQUID Na/Ca EXCHANGE.

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Metabolic up-regulation of the Na/Ca exchanger by ATP transforms and inefficient carrier into a highly efficient one by increasing the affinities for Ca_i and Na_o. The mechanism for ATP regulation is different among species and even for different tissues of the same species. Nevertheless, a common property is that a phosphorylation dephosphorylation process is involved. Three steps will be considered: i)the pathway for phosphoryl group transfer: a protein or a lipid kinase (s). ii) the target for phosphorylation: the exchanger or other regulatory molecule/s, and iii) the dephosphorylation process. For the squid Na/Ca exchanger the data show: 1)ATP modulation does not involve the classical protein kinases (PKA, PKC, PtyrK, CAM-PK). 2) a soluble 13 kDa cytosolic regulatory protein (a novel protein) is required, 3)deactivation of the Na/Ca exchanger by removal of ATP occurs through a Mg^{2+}i-dependent process, and 4) phosphoinositides are not involved. Finally, we will present evidences for a novel metabolic pathway of regulation of the squid exchanger by phosphoarginine. (CONICIT S1-97001675; CONICET 4904/97, FONCYT 05-00000-01092/97, NSF IBN-9631107).

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HOW OUABAIN WORKS: KEY ROLE OF THE PLASMEROSOME IN THE REGULATION OF CELL RESPONSIVENESS. ((**M.P. Blaustein**, A. Arnon, J.M. Hamlyn and M. Juhaszova)) Physiol. Dept., Univ. Maryland Med. Sch., Baltimore, MD 21201

Cardiotonic steroids are believed to amplify cell activity by inhibiting Na^+ pumps and elevating cytosolic Na^+ ($[Na^+]_{CYT}$). Na^+ pump catalytic subunit (α) isoforms with different affinities for ouabain and Na^+ distribute differently in the plasmalemma (PL) of rat astrocytes, neurons, and arterial myocytes. The low ouabain affinity (IC_{50} >10,000 nM), high Na^+ affinity ($K_d \approx$ 12 mM) isoform (α1) is ubiquitously distributed in the PL, but high ouabain affinity (IC_{50}< 50 nM), low Na^+ affinity (K_d's $\approx$ 24-33 mM) isoforms (α2 and α3) are confined to PL domains overlying sarcoplasmic/endoplasmic reticulum (S/ER). This implies that the different isoforms have distinct functions. The junctional S/ER and adjacent PL form a functional unit ("PLasmERosome") containing $\approx 10^{-12}$-10^{-13} liters cytosol. We tested 10-100 nM ouabain on evoked Ca^{2+} (Fura-2) transients in rat artery myocytes. Transients evoked by 30 s of 5-HT, AVP, or S/ER Ca^{2+} pump inhibitors, were augmented by 30-120 s exposure to ouabain, human ouabain-like compound, or 1 mM K^+; "bulk" resting $[Na^+]_{CYT}$ (SBFI) and $[Ca^{2+}]_{CYT}$ were not elevated. Augmentation required external Na^+ but not Ca^{2+}; removal of external Na^+ for 120 s augmented evoked Ca^{2+} transients in the absence of ouabain. *Conclusion*: α2/α3 Na^+ pumps control PLasmERosome $[Na^+]_{CYT}$; this, in turn, regulates, via Na^+/Ca^{2+} exchange, local $[Ca^{2+}]_{CYT}$, Ca^{2+} signaling and cell responses.

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Ca/H EXCHANGE VIA THE PLASMA MEMBRANE Ca ATPase IN SKELETAL MUSCLE

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The aims of this work were to determine: 1) whether Ca exit via the plasma membrane Ca ATPase is coupled to H entry (Ca/H exchange); 2) whether operation of the Ca ATPase has an absolute requirement on external H; 3) the stoichiometry of the Ca/H exchange; and 4) whether the Ca/H exchange is voltage-sensitive. The internally-perfused, voltage-clamped barnacle muscle cell was used as a model. The results indicate the following: i) Ca efflux is dependent on external pH, is accompanied by a membrane depolarization and an intracellular acidification that is greater than that expected from Ho "leak" and ATP hydrolysis; ii) Ca efflux is inhibited in the presence of various plasma membrane Ca ATPase blockers and by ATP depletion, indicating that a plasma membrane Ca ATPase mediates Ca/H exchange; iii) Ca/H exchange is not absolutely required for operation of the plasma membrane Ca-ATPase; iv) Ca/H exchange is voltage-sensitive being activated by depolarization; and v) the coupling ratio of the Ca/H exchange varies depending on the availability of Ho. At an acidic pHo (6.5), the plasma membrane Ca ATPase mediates a fast Ca/H exchange with a coupling ratio of 3-4 H: 1 Ca whereas at an alkaline pHo (8.2), it operates slowly with a likely coupling ratio of 0 H/1Ca. It is proposed that the plasma membrane Ca ATPase mediates a voltage-sensitive Ca/H exchange with a variable stoichiometry

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THE THYROID IODIDE TRANSPORTER: THE EMERGING MOLECULAR PICTURE

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The Na^+/I^- symporter (NIS), a 618 amino acid membrane glycoprotein that mediates the active accumulation of I^- into thyroid cells, has been identified, characterized at the molecular level in our laboratory, and analyzed electrophysiologically. We have substituted both separately and simultaneously the asparagine residues in all three putative N-linked glycosylation consensus sequences of NIS with glutamine, and assessed the effects of the mutations on function and stability of NIS in COS cells. All mutants were active and displayed 50-90% of wild-type NIS activity, including the completely non-glycosylated triple mutant. This demonstrates that function and stability of NIS are preserved in the absence of N-linked glycosylation. We also found that N225 is glycosylated, thus proving that the hydrophilic loop that contains this amino acid residue faces the extracellular milieu. We demonstrated that the NH_2 terminus faces extracellularly as well. A new secondary structure model consistent with these findings has been proposed. Patients with congenital lack of I^- transport do not accumulate I^- in their thyroids, often resulting in severe hypothyroidism. A single amino acid substitution in NIS, proline replacing threonine at position 354 (T354P), was recently identified as the cause of this condition in several independent patients. Here we report that in T354P NIS the lack of I^- transport activity is not due to a structural change induced by proline, but rather to the absence of a hydroxyl group at the b carbon at position 354. Hence, this hydroxyl group is essential for NIS function